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Chapter 13

Vaginal Formulations for Prevention of Sexual Transmission of HIV

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Abstract

According to UNAIDS, as there is still no effective vaccine against HIV, pre-exposure prophylasis (PrEP) is necessary to reduce its incidence. Sexual transmission rate is higher from men to women in developing countries and vertical transmission may also occur from mother to child. Hence, vaginal formulations are an interesting proposal for the protection of women, preventing the virus from infecting vagina through different mechanisms. Several drugs, such as Dapivirine, Tenofovir or Maraviroc, have been assessed and showed to be effective in this field. These microbicides are included in different dosage forms able to release the drug once in contact with the vaginal medium. Innovative excipients are being employed for the development of different systems trying to get an easier posology through control release and high comfortability, thus leading to a better compliance. In this line, several formulations have been developed and tested, such as rings, tablets, gels or films. Some of them are nowadays in clinical trials, such as a Tenofovir gel or a Dapivirine vaginal ring. The aim of this chapter is to synthetize the research and findings in the field of the development and assessment of vaginal formulations in the PrEP of HIV sexual transmission.

Keywords: HIV sexual transmission prevention, vaginal gels, vaginal tablets, vaginal films, vaginal rings

1. Introduction

It is well known that the human immunodeficiency virus (HIV) is one of the most serious epidemics facing humanity today, when 36.7 million people live with HIV. However, excellent

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progress has been made in access to antiretroviral therapy in the last decade, reducing acquired immunodeficiency disease syndrome (AIDS)-related deaths by 48% [1]. Prevention programmes have also succeeded in lowering the transmission of the virus in recent years, although it still continues to cause 1.8 million new infections per year.

One fact that gives pause for thought is that most of the people infected with HIV are women. The data is devastating, as AIDS-related diseases continue to be the leading cause of death among women of reproductive age, and it is also the second leading cause of death in Africa for women aged 15–24 [1]. The gender gap is much more pronounced among young people: in the 15–24 population group, new infections are 44% higher in women [1]. In light of these figures, there is no doubt that the risk of infection in young women is unacceptably high.

These already chilling figures are even more shocking in women living in sub-Saharan Africa, where 75% of new infections in the population aged 15–19 occur in girls [2]. This is also a population group where the incidence of new infections has barely diminished in recent years.

Although in theory their lower access to antiretroviral therapy might appear to be the main reason for the high prevalence of HIV among sub-Saharan women, a careful analysis of the living conditions in this area reveals that the high transmission rate of the virus cannot be attributed to a single reason. It is important to bear in mind that this is an area where rape and domestic violence are frequent. The World Health Organization (WHO) states that women living in these conditions are 50% more likely to acquire HIV [2].

However, the most important reason for this prevalence is undoubtedly women's lack of access to education and economic independence, which prevents them from negotiating the possibility of having safe sex with their partners. The stark reality is that 75% of young women do not have the final say over their own health [2]. To this must be added polygamy, which is still common in several sub-Saharan countries (Burkina Faso, Congo, Ivory Coast, Ethiopia, Gabon, Guyana, Rwanda, South Africa, Uganda, Tanzania and Zimbabwe) and the decrease in the use of condoms registered in Ivory Coast, Niger, Senegal and Uganda [3].

A cycle of HIV transmission has recently been described in sub-Saharan Africa to explain the negligible decline in the prevalence of AIDS in this area compared to the rest of the planet. Adult men typically infect young women (each year 15 million women are married before the age of 18). Later, when the women grow older, they tend to transmit the virus to men of their own age, who then start the cycle again [2].

This reflection points to the empowerment of women, especially in sub-Saharan Africa, as a fundamental milestone for halting the AIDS epidemic. It is essential to promote societies in which gender equality is achieved through adequate sexual and social education, and the aim of the United Nations (UN) is to ensure that young people have the skills, knowledge and tools to protect themselves from acquiring the virus [2]. However, this capacity for protection must be reinforced by empowering women and providing them with methods for preventing the sexual transmission of HIV that are not dependent on men, such as vaginal microbicides. This is the reason that research into vaginal microbicides has skyrocketed in recent decades, which, if they prove to be successful in preventing HIV, would represent an extraordinary step forward in the fight against AIDS.

Vaginal microbicides have been defined as "any agent included in a topical formulation designed to prevent the spread of sexually transmitted pathogens either through cell death, inactivation of cell mechanisms, inhibition of viral replication, the formation of a physical barrier between cells and pathogens, or by enhancing the natural protection mechanisms of the cervix and vagina" [4].

The strategies for developing an effective vaginal microbicide in recent decades have been so diverse that they require a preliminary classification to aid their understanding. They can initially be divided into two groups depending on whether the microbicides include antiretroviral drugs or not.

Microbicides that do not include drugs can be differentiated into surfactants, polyanions, acidifiers and glycoprotein 120 neutralizing monoclonal antibodies. The aim of these substances is to inactivate the virus before it meets the cells so the infection never occurs.

Microbicides that include antiretroviral drugs can be classified into entry inhibitors or viral enzyme inhibitors [4]. Of particular interest in this group is Tenofovir, an inhibitor of the reverse transcriptase of the virus, which was part of the first microbicide that proved its effectiveness in preventing the transmission of HIV, and has now become the most widely-studied drug for this purpose [3, 5]. Dapivirine, one of the most promising drugs for the development of vaginal microbicides against HIV, has subsequently also become very important [6].

The development of microbicides has evolved over the years. The initial conventional release formulations did not usually include antiretroviral drugs. Over time, the potential of useful antiretroviral drugs for preventing HIV infection use was assessed, and microbicides including different drugs gradually began to appear. The possibility of developing a microbicide that does not contain one of these antiretroviral drugs is now hardly contemplated, and the new trend in microbicides is to develop formulations for sustained drug release for more lasting protection [7].

Unfortunately, the vast majority of microbicide formulations developed to date have failed to afford protection due to their low efficacy or inadequate formulation [8]. This is often due to a failure to consider the characteristics of the vaginal route.

The main anatomical factor to bear in mind when developing a formulation for vaginal administration is the vaginal fluid, which can be both an ally and an enemy for our purpose. This aqueous fluid is produced from the mucous membranes of the endometrium and transudate serum, and accumulates inside the vagina and covers the vaginal epithelium [9]. The physical presence of this fluid and its high enzymatic activity has been identified as barriers to the release and absorption of drugs. It should also be noted that this fluid is generally the medium in which the drug must be dissolved, meaning that its components are highly likely to interfere in the drug's activity [10]. To make matters worse, it is also necessary to factor in dynamic changes in the volume and composition of this fluid, since the vaginal clearance of drugs administered by this route is also crucial for their efficacy [11].

pH also plays a significant role in the effectiveness of the formulations. The normal pH of vaginal fluid is between 4 and 4.5, while the pH of seminal fluid is around 7.9 [12]. Even if a formulation were to be developed that was effective in the pH of the vaginal environment, the microbicide could potentially lose its protective capacity in the presence of seminal fluid, when the medium undergoes alkalization.

Another similar example of failure due to the natural characteristics of the administration route is the collapse of certain microbicides (those that include polyanions) in *in vivo* trials, despite their success in blocking HIV *in vitro*. It was subsequently discovered that this inefficacy was due to the formation of a semen-derived enhancement of the virus infection that increased the infectious capacity of the virus in the presence of seminal fluid [13]. This study highlights the importance of evaluating the efficacy of microbicides in the presence of semen [4].

Another factor that must be considered when developing a microbicide is that the vaginal epithelium and the mucus layer covering it is already a barrier against infections. There is therefore an obvious need to maintain this natural barrier intact when seeking to prevent the acquisition of sexually transmitted diseases. A negative example of this is the microbicidal gel including Nonoxynol-9, a surfactant, which was shown in clinical trials to lead to an increase in vaginal ulcers [14].

Pharmaceutical form	**Advantages**	**Drawbacks**
Gels	• Widely studied and well known. • Easy and convenient for women to apply. • Low manufacturing cost and easy to mass produce.	• Unable to retain the drug and provide sustained release. • They require an applicator for administration • Possible local irritation and leakage. • Not particularly stable against adverse environmental conditions.
Tablets	• Easy and economical to manufacture on an industrial scale. • Easy to handle • Stable under different environmental conditions • Fast-dissolving or sustained-release tablets can be obtained depending on the excipients used in their development.	• Possible influence on sexual intercourse. • Possible local irritation.
Films	• Discreet use • No product leakage during use • No applicator required for insertion • Minimal packaging and reduced waste.	• Sustained release still not achieved • Possible local irritation • Mass production is currently unviable due to the underdevelopment of production resources.
Vaginal rings	• Sustained release of the drug. • Fewer applications. • The mass production of this dosage form is becoming increasingly advanced.	• They require a higher financial investment. • Higher manufacturing cost. • Possible influence on sexual intercourse.

Table 1. Advantages and disadvantages of pharmaceutical forms for vaginal administration of microbicides.

Another crucial factor is the vaginal microflora, which plays a very important role in establishing the microbicide's eventual environment. Special care should be taken with commensal bacteria, which are responsible for maintaining a healthy vaginal environment. Vaginal microbicides must therefore not be toxic to the vaginal microbiota [10].

The most common pharmaceutical dosage forms for the vaginal administration of drugs are gels, capsules, ovuli and tablets, although vaginal rings and films are rapidly gaining ground [4]. The first trials of vaginal microbicides mainly explored vaginal gels, but the current trend focuses more on vaginal rings and sustained-release tablets, which could prolong protection time. The advantages and drawbacks of each dosage form are summarized in **Table 1**.

Advanced drug delivery strategies are being incorporated for drug targeting, together with scientific methods to develop safer and more effective formulations [15]. Current research therefore gives cause for hope that within a few years women will be able to protect themselves from HIV acquisition through vaginal microbicides.

2. Main vaginal systems for HIV prevention

2.1. Vaginal gels

2.1.1. Overview

Gels are semisolid systems consisting of a liquid—generally water—and a solid component that acts as a gelling agent and traps the liquid within its three-dimensional structure, thus producing the characteristic consistency of these solid–liquid mixtures [16] (**Figure 1**). Vaginal gels can release drugs intended for local or systemic action [17]. However, the direct application of drugs onto the vaginal epithelium limits their systemic absorption, thus minimizing side effects and possible drug resistance [15, 18].

An ideal vaginal microbicide gel should be as spreadable as possible in order to cover the entire vaginal surface mucosa and thus protect effectively against the transmission of the virus. Several authors have pointed to an influence of the gel composition—which determines its viscosity and rheological properties—in its ability to form a stable coating layer. In addition to their composition, parameters such as the pH and osmolality of these gels must be suitable for the characteristics of the vaginal environment in order to achieve the microbicide effect without inducing side effects that could increase the risk of infection and/or result in the patients' loss of adherence [15, 19, 20].

One of the main problems associated with the vaginal administration of gels—especially those intended for the controlled release of drugs—is the low retention of the formulations at the site of action due to the effect of gravity and clearance by vaginal fluids. This causes a loss in the formulation and subsequent under-dosing, leading to low therapeutic efficacy—as more frequent administration is required—and low acceptability by patients [15, 21].

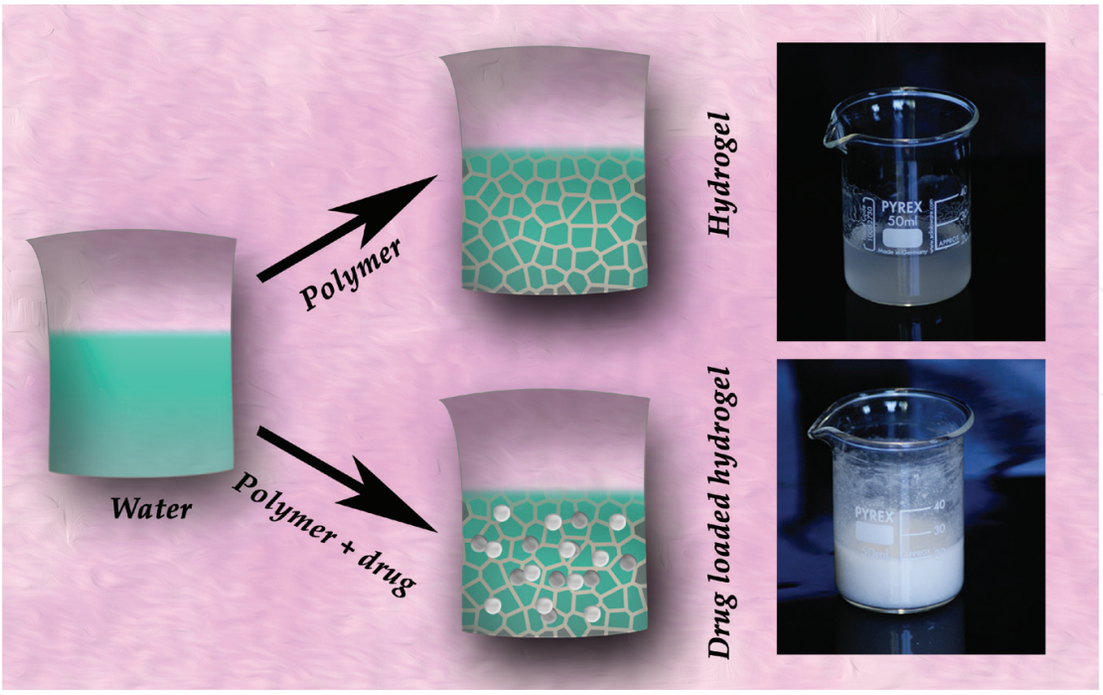

Figure 1. Process of obtaining a hydrogel and a drug-loaded hydrogel.

Two main approaches have been proposed to overcome this problem. One is to develop **mucoadhesive formulations** that remain attached to the vaginal surface for longer and allow a controlled release of the drugs they contain. These mucoadhesive properties are based on the interaction between the dosage form and either the secreted mucus or the mucosal membrane and are usually obtained by including polymers in the formulation. The mucoadhesive polymer chains create bonds—mainly van der Waals and hydrogen bonds or electrostatic interactions—with the mucins in the mucus. Polymers containing numerous functional groups, like hydroxyl or unionized carboxylate groups, are promising excipients for mucoadhesive formulations. Polyacrylic acid derivatives such as carbomer and polycarbophil, cellulose derivatives like hydroxyethyl cellulose (HEC), hydroxypropylmethyl cellulose (HPMC) and carboxymethyl cellulose (CMC), chitosan, hyaluronic acid, alginate, carrageenan and gums are therefore some of the most widely used mucoadhesive polymers in vaginal formulations [15, 19, 20, 22].

The second approach is the use of **thermogelling formulations**. These are *in situ* gel-forming systems which gel when the temperature exceeds a specific gelation value. Systems which gel between room temperature and physiological temperature are very useful in vaginal formulations. Their low viscosity before application means that thermogelling formulations offer a higher spreadability, resulting in the formation of a resistant gel layer in the vagina, a very important aspect for microbicide systems [15, 20]. Most vaginal thermogelling systems contain polymers that can gel at the physiological temperature and are also mucoadhesive. Poloxamers are a group of triblock copolymers composed of polyethylene oxide, polypropylene oxide and polyethylene oxide that are widely used in aqueous solutions in concentrations higher than a critical value for the manufacture of thermogelling systems. Gelation occurs when the poloxamer molecules aggregate to form micelles. However, poloxamer gel has low mucoadhesion properties, so thermogelling systems based on these polymers have been combined with

mucoadhesive ones. Another polymer widely used in these *in situ* gel-forming formulations is chitosan, in combination with polyol salts like β-glycerophosphate (GP) or glyceryl monooleate. The thermogelling of chitosan/GP mixtures occurs through the loss of hydration water from the polymer chains and the subsequent strengthening of the hydrophobic interactions as the temperature increases. Other authors point to different interactions such as electrostatic attraction between the ammonium groups of chitosan and the phosphate group of GP, hydrogen bonds between chains, and hydrophobic interactions by reducing electrostatic repulsion of polymer chains, as the mechanisms responsible for the gelling process [20].

2.1.2. Vaginal gels for HIV prevention under development

Forbes *et al.* developed a non-aqueous silicone elastomer gel containing Maraviroc as a hydrophobic anti-HIV agent and compared it to a 2.2% w/w HEC gel. It was subjected to different assays, including *in vitro* placebo gel retention, *in vitro* drug release and pharmacokinetic studies in rhesus macaques. The silicone gel showed a longer retention time, a slower rate of Maraviroc release in simulated vaginal fluid and a higher and more sustained drug concentration in vaginal fluid, vaginal tissue and plasma than the HEC gel [23].

Li *et al*. proposed a thermosensitive hydrogel of methyl cellulose modified by stearic acid (MCS) in presence of sodium chloride and phosphates. The gelation process occurred at the physiological temperature and at an even lower value. *In vitro* cytotoxicity tests and *in vivo* evaluation of mucosal irritation attributed good biocompatibility properties to the MCS hydrogel, which also showed a sustained release of Tenofovir for 10 h without any burst effect [24].

In the field of nanotechnology, Lara *et al*. formulated polyvinylpyrrolidone-coated silver nanoparticles (PVP-coated AgNPs) in a concentration of 0.15 mg/mL in the non-spermicidal Replens gel [25]. These PVP-coated AgNPs had previously demonstrated anti-HIV activity [26]. The resulting gel containing the nanoparticles showed an inhibition of HIV-1 transmission after 1 min and offered prolonged protection for 48 h in human cervical cultures. This formulation was not found to be cytotoxic at the aforementioned concentration of PVP-coated AgNPs during the 48 h of protection [25]. Another example is the work of Date *et al*., who developed a thermosensitive vaginal gel with poly(lactic-co-glycolic acid)—PLGA—nanoparticles loaded with Raltegravir and Efavirenz to prevent HIV infection. The mean encapsulation efficiency of the drugs was 55.5 and 98.2%, respectively. Two poloxamers (Pluronic® F127 and Pluronic® F68) were used to obtain the thermogelling system, which gelled at 32.5°C. The drug- loaded nanoparticles allowed the sustained intracellular release of Raltegravir and Efavirenz in HeLa cells and the cytotoxicity assays indicated that these particles or the blank gel were not toxic in these cells for the 14 days of the study, compared to control cells without treatment [27].

Some of the clinical trials currently underway on vaginal gels for HIV prevention are shown in **Table 2**.

2.2. Tablets

2.2.1. Overview

Vaginal tablets are solid monolithic matrix systems designed to be placed in the vagina and release the drug in this area. Since several vaginal tablets are already marketed in developed

Study	Phase	Formulation	Reference
MTN-004	I	3% w/w SPL7013 gel (VivaGelTM)	[28]
Population Council #558	I	PC-1005 (MIV-150/zinc acetate carrageenan gel)	[29]
CAPRISA 004	IIb	1% Tenofovir gel	[30]
Population Council #322	III	Carraguard®(PC 515) gel	[31]
C03-090	III	6% cellulose sulphate gel	[32]
FACTS 001	III	1% Tenofovir gel	[33]

Table 2. Vaginal gels for HIV prevention under clinical investigation.

and developing countries for the treatment of different diseases, such as vaginal atrophy, vulvovaginal candidiasis and bacterial vaginosis, tablets may represent a potential alternative for the formulation of microbicides [34].

The advantages of vaginal tablets include their higher dose accuracy and greater stability than semisolid dosage forms, and the fact that they are easy and cheap to manufacture [34, 35]. These formulations are also versatile as they can be used for immediate or controlled release, the first of which is achieved by rapid disintegration of the system using disintegrants such as crospovidone as excipients [36]. Nevertheless, controlled release systems must contain mucoadhesive polymers as the main excipients. These polymers need to interact with the vaginal surface through the interrelation of certain specific chemical groups in the polymers and biological tissues for the formulation to remain attached to the vaginal mucosa while the drug is being released, which is a critical factor [37]. The gelation process of these polymers in contact with aqueous media may be useful for controlling the release of the drug via diffusion through the gel layer (**Figure 2**). Some examples are cellulose derivatives, guar gum and chitosan [38].

However, the disadvantages of tablets include comfort issues such as difficulty in self-insertion and their slow rate of disintegration [39]. Contact between the mucosa and the solid formulation may provoke vaginal irritation. Vaginal hydration must also be increased to ensure the correct distribution of the drug over the whole vaginal surface [15].

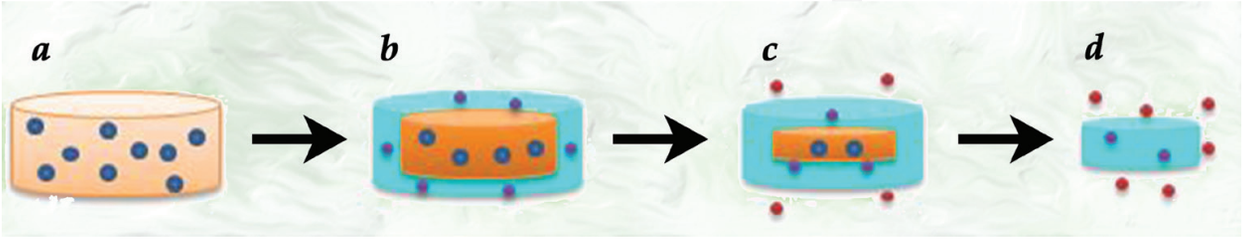

Figure 2. Drug release through tablets. Tablet after vaginal application (**a**). Formation of a drug-loaded gel in contact with the vaginal fluid (**b**). Drug diffusion through gel layer reaching vaginal environment. Erosion of external gel layers (**c**). Complete transformation of tablet into a gel; Total drug diffusion and gel erosion (**d**).

2.2.2. Vaginal tablets for HIV prevention under development

Immediate-release tablets containing Emtricitabine and Tenofovir as antiretroviral drugs were developed by Clark *et al*. Tablets containing disintegrants were prepared to produce immediate release in contact with vaginal fluid. Maximum concentrations in vaginal tissues and fluid were observed less than 1 h after administering the tablets to rabbits, so the drug distribution and release results were comparable to those observed in a clinical trial with a gel containing 1% TFV [34].

Immediate-release tablets have also been explored for the development of biotherapeutic assets, including MucoCept, the name for human vaginal *Lactobacillus jensenii* that has been genetically modified to express the HIV entry inhibitor, modified cyanovirin-N (mCV-N). These bacteria were mixed with various excipients and freeze-dried, obtaining fast-dissolving highly hydrophilic tablets. Samples were assessed *in vitro* and *in vivo* using macaques. The tablets showed complete disintegration in 2 min and the colonization of the vaginal mucosa occurred in up to 83% of the macaques in 21 days [40].

Some attempts have been made to achieve controlled drug release, but several factors must be improved to overcome vaginal leakage and obtain a more comfortable posology. McConville *et al.* developed multilayer tablets based on Kollidon® containing a combination of Acyclovir, Levonorgestrel and Dapivirine. These systems released the first two drugs immediately, and Dapivirine release was sustained for more than 8 h [36]. Nevertheless, daily administration would still be necessary.

Innovative formulations that have shown efficient controlled drug release are based on the addition of hydrophilic mucoadhesive polymers that form a gel in an aqueous medium such as the vaginal environment. The formulation remains attached to the mucosa and forms a strong gel that may lead to a the sustained diffusion of the drug, slowing its rate of release. This was demonstrated in research by Notario-Pérez *et al.*, where compacts based on a combination of chitosan and hypromellose proved their ability to gel in simulated vaginal fluid, and the gel obtained *in situ* produced the sustained release of Tenofovir in this medium, with high mucoadhesion [35]. These hydrophilic matrices have also been improved with the addition of hydrophobic granules of Eudragit® and zein containing the drug. This modification led to longer periods of release and mucoadhesion through complex release mechanisms, thus obtaining compacts suitable for the controlled release of Tenofovir over 6 days [7].

2.3. Films

2.3.1. Overview

Films are thin, soft, flexible sheets obtained by the solvent-casting method (**Figure 3**) or—less frequently—by hot-melt extrusion. Films are typically designed to disintegrate within a few minutes on contact with vaginal fluids, so the drugs are released promptly [41, 42]. Vaginal films are well accepted by women as they are discreet and easy to use and carry around due to their low weight and size, and suitable for the design of new microbicides [39]. They also have good physicochemical stability and resistance to microbial contamination.

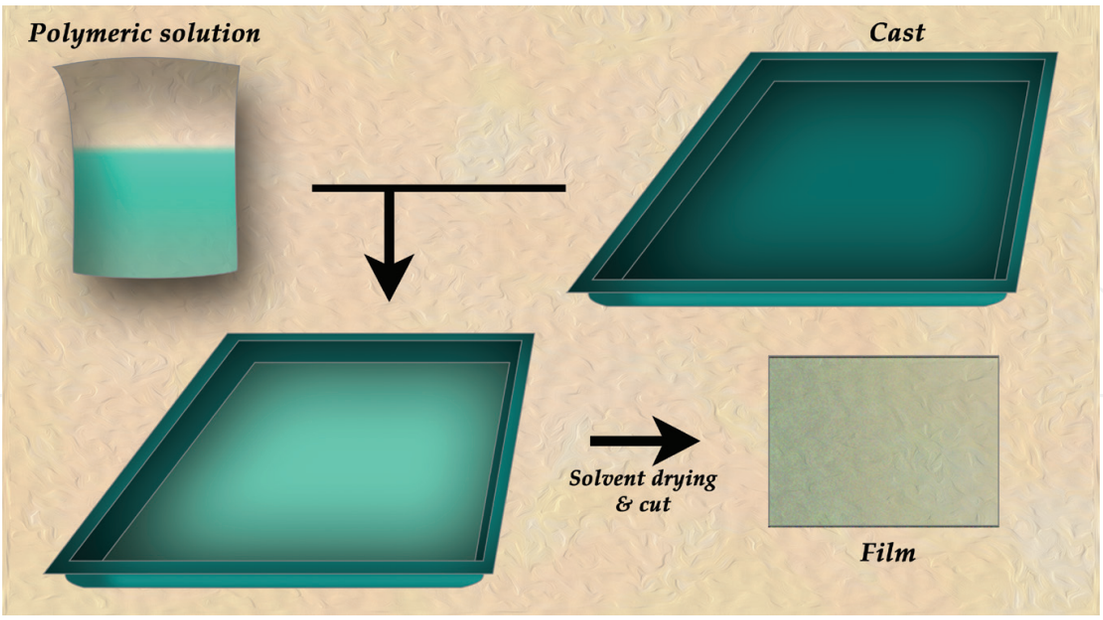

Figure 3. Solvent casting method for manufacturing films.

Matrix-forming polymers give the film mucoadhesive properties, which prevents vaginal leakage and improves drug release. Films are inexpensive to manufacture and can easily be reproduced, and their stable solid formulation makes them suitable for the release of drugs.

However, films also have some disadvantages that must be taken into account when developing these systems. Due to their small size and weight they can incorporate only a small amount of drug, generally less than 50% of the total weight of the formulation [42]. The limited volume of vaginal fluid may condition the disintegration process and hence the release of the drug. Another factor to consider is the wide variability in vaginal fluid secretion among different women and even at different times, making the release sometimes difficult to predict [43]. Administration may also be problematic, as the films start disintegrating immediately the come into contact with vaginal fluid [41]. Lastly, the mechanisms for regulating the manufacture and characterization of films are not yet sufficiently standardized [44].

Immediate-release films have been widely explored, and fast-dissolving products are generally used as matrix-forming polymers, which significantly influences the properties of the final product. These polymers cannot be toxic or irritant and must form films with good wetting properties and disintegration time and high tensile strength. Polyacrylates, polyethylene glycol, polyvinyl alcohol (PVA) and cellulose derivatives are currently the polymers of choice to formulate vaginal films. Plasticizers such as glycerol or polyethylene glycols are also included to give the film flexibility and facilitate handling and administration [44]. Other components are frequently included in addition to matrix-forming polymers and plasticizers; for example preservatives or disintegrants [42]. Work is currently underway to obtain controlled release and long-term efficacy and stability [45]. The inclusion of nanoparticles may also lead to promising drug delivery systems [46]; these thin polymer-based films could serve as platforms for the administration of nanosystems, as they overcome several of the disadvantages of other systems (nanoparticles alone or nanoparticles containing gels). For instance, the vaginal leakage observed in previous nanoparticle systems (free or contained in

a gel) is reduced due to the high mucoadhesion of the hydrated film which forms polymers. They also prevent the premature release of the drug from nanoparticles to the aqueous phase of the gel that occurs during storage. Other advantages include reduced discomfort and easier incorporation and release of the nanosystems compared to other solid formulations (such as rings and tablets) [41].

2.3.2. Films for HIV prevention under development

HPMC was used as the main excipient in the development of films containing mixtures of Dapivirine, Tenofovir and/or Maraviroc by the solvent casting method. Other excipients were added as secondary polymers (PVA or CMC) and plasticizers (glycerol or polyethylene glycol 800). This study demonstrated the versatility of the formulation according to the polymers used when loading drugs with different solubilities. More than half of each drug was released within the first 30 minutes of application, thus serving as an option for pericoital prophylaxis. These films showed HIV activity for up to 1 year [47].

PVA -based films have also been used as formulations for the release of new drugs: Sassi *et al*. developed a fast-dissolving Retrocyclin-analogue film that showed *in vitro* and *ex vivo* activity against HIV [48].

HPMC has also been combined with sodium alginate for the development of Abacavir-based films containing glycerol as a plasticizer. The film gels in contact with vaginal fluid to obtain a highly bioadhesive formulation. More than half of the drug was released in the first 30 minutes, making this another interesting option for pericoital prevention [45].

Akil *et al.* combined HPMC with PVA and polyethylene glycol 8000 for the administration of Dapivirine. Again, an immediate-release formulation was obtained for the prevention of HIV, with no apparent toxicity for the vaginal microbiota. Good stability properties were also observed, and anti-HIV activity persisted for at least 18 months [49].

The use of antimicrobial products has also been proposed as film-forming agents. Garg *et al.* developed bioadhesive vaginal films of sodium polystyrene sulfonate. Their results suggested that these films could have interesting biological, pharmaceutical and esthetic properties and may offer substantial benefits for preventing the sexual transmission of HIV [50].

Lastly, films are currently an option for the incorporation of nanoparticles. Srinivasan *et al.* prepared vaginal films containing a novel non-nucleoside reverse transcriptase inhibitor—IQP0528—with and without PLGA-Eudragit® S 100 nanoparticle encapsulation of the drug. Both formulations released a higher amount than required to exceed IC50 and showed no toxicity or alterations in vaginal microbiota. The drug release profile from nanoparticle-based films was some orders of magnitude greater than for films containing the free drug [42].

Cunha-Reis *et al*. developed fast-dissolving HPMC/PVA-based films containing nanoencapsulated Efavirenz in PLGA nanoparticles and free TFV. They were characterized *in vitro* and *in vivo* and the results from mice revealed that concentrations of the drug in vaginal secretions decreased rapidly after administration, and were more pronounced for the free than for the encapsulated drug. However, AUCs for both drugs were some orders of magnitude higher than obtained when administering both drugs in an aqueous vehicle. Films therefore represent an option for drug administration due to their longer residence time in the vagina. It

Study	Phase	Formulation	Reference
FAME-02	Phase I	Dapivirine vaginal film and gel	[52]
FAME-05	Early Phase I	Tenofovir vaginal film and gel	[53]

Table 3. Vaginal films for HIV prevention under clinical investigation.

was also demonstrated that nanoparticles can prolong the release of the drug, indicating that nanoparticle-based films have potential for the development of controlled release systems [51].

Table 3 shows the vaginal films for HIV prevention currently under clinical investigation.

2.4. Vaginal rings

2.4.1. Overview

Vaginal or intravaginal rings are dosage forms placed closed to the cervix [54, 55] and designed to release drugs in a controlled or sustained manner over weeks or months to achieve either local or systemic action. From a physicochemical point of view, they are flexible toroidal devices with a polymeric structure. The first references to vaginal rings for drug delivery date from the late 1960s and 1970s with a patent from the Upjohn Company and the discovery that several drugs, including steroids, could be released through silicone elastomers [55–57]. The first clinical evaluation of a vaginal ring—for contraceptive purposes—took place in the 1970s [57]. The inclusion of microbicides against HIV in vaginal rings was proposed at a microbicide conference in 2002. The first article on microbicide vaginal rings for HIV prevention was published 1 year later, and described a matrix-type silicone elastomer vaginal ring with *in vitro* release of Nonoxynol-9 over 8 days. This drug subsequently ceased to be seen as a microbicidal candidate as its gel formulation increased the risk of HIV transmission due to the damage to the vaginal epithelium. In 2005, a core-type silicone elastomer vaginal ring was described which released Dapivirine (or TMC120) *in vitro* over the 71 days of the study, and was attributed a sustained release of the total dose for between 1 and 4 years [54–56].

Vaginal rings with microbicide formulations are well accepted by women as they can be inserted and removed by the patients themselves, they offer controlled release of the drugs—resulting in a convenient dosage regimen—and are compatible with the sexual act, all of which ensures greater therapeutic compliance and effectiveness [54–57].

Two main types of polymers are used in the development of vaginal rings: silicone and thermoplastic elastomers. Silicone elastomers are obtained by chemical crosslinking of functionalized, linear, polydimethylsiloxane [56]. Thermoplastic elastomers have rheological properties which alter during heating (flow) and cooling (harden). The most important elastomers for the manufacture of vaginal rings are polyurethanes (PUs) and ethylene and vinyl acetate (EVAs) copolymers [54, 55]. The vinyl acetate content (which ranges from 10–40% approximately) and the molecular weight of the EVAs influence the mechanical properties and the drug release rate of the final formulation. Although there are few cases of EVA vaginal rings for microbicide release [54, 56], the possibility of varying the vinyl acetate content of EVA materials offers a broader range of drug permeation properties than silicone elastomer materials, which

are also more expensive. EVA polymers can also form rate-controlling membranes that are thinner than 100 μm. PUs are bi-phasic copolymers that constitute alternative biocompatible materials and are also under study for the development of microbicide vaginal rings. They are obtained by a polymerization reaction between diisocyanates, a low molecular weight diol and a high molecular weight diol. The combination of the diisocyanate with the low molecular weight diol leads to the formation of hard segments—responsible for the material's resilience—while combination with the high molecular weight diol produces soft segments which confer elasticity and elastomeric properties. By varying the proportion of hard and soft segments, hydrophobic and hydrophilic PUs can be obtained to allow the optimal release of different types of drugs [56].

Vaginal rings come in different designs (**Figure 4**). **Spring vaginal rings** were the first to appear and were formed by a metal spring over-molded by a silicone sheath. Despite offering good results in terms of their clinical efficacy, they were also rigid and had several adverse effects [56]. **Matrix or homogeneous vaginal rings** have the simplest design and consist of a polymeric matrix in which the drug is dispersed. Drug release occurs through a permeation mechanism involving the dissolution of the drug in the polymer and diffusion through the matrix. The delivery rate depends on the solubility of the drug in the polymer, the ability of the drug to diffuse through it, the drug content and the surface area of the ring [54, 55]. Until 2006, **reservoir vaginal rings** and matrix rings were the main microbicide vaginal ring designs. Also known as "core vaginal rings," they are formed by a polymer membrane which encapsulates one or more cores loaded with the drug. The possibility of including several cores in one reservoir ring ("multi-core ring") allows the formulation of different drugs in the same dosage form [55, 56]. The cores can also be inserted in a ring before sealing the ends [54]. Drug release also occurs through permeation and can be controlled by modifying the thickness of the membrane [55, 56]. **Segmented vaginal rings** are formed by two or more connected segments loaded with one or more drugs; they allow control over the release of each drug and avoid interactions between them [54, 55]; they may be either matrix- or reservoir-type systems [56]. **Sandwich or shell vaginal rings** are based on an outer membrane, a narrow drug-loaded

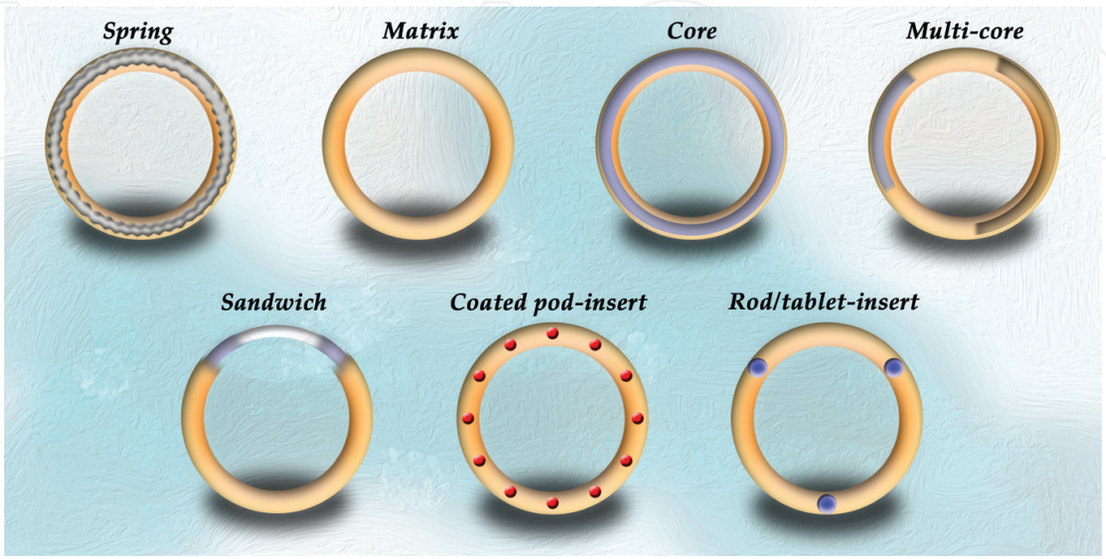

Figure 4. Vaginal ring designs.

polymer layer and an inner central core. As in the case of reservoir rings, the release rate depends on the thickness of the outer membrane [54, 55]. In order to overcome the problems of permeability when releasing hydrophilic and/or high molecular weight drugs from vaginal rings based on silicone or thermoplastic elastomers, several new designs of these dosage forms have been developed. **Coated pod-insert vaginal rings** are silicone elastomer rings containing drug cores or pods coated with layers of polymers such as semipermeable polylactic acid. The drug release rate depends on the size of the delivery window in the silicone ring, the number of cores and the amount and composition of the core coating. This ring design allows the release of drugs with different physicochemical properties from the same dosage form. **Rod and tablet-insert vaginal rings** are silicone elastomer rings containing freeze-dried polymer gel rods or compressed polymeric tablets with the drug/s. The gel reconstituted by vaginal fluid and the tablet both release the drug in a sustained manner. The lyophilized gel rods are interesting for the formulation of peptide- and protein-based microbicides, as the freeze-drying process stabilizes this kind of drug. **Biosoluble and hydrogel vaginal rings** are other new designs of vaginal rings. For instance, biosoluble acacia gum, nonbiodegradable hydrogels based on methacrylates and a nanoporous polydiol citrate elastomer hydrogel have been used to manufacture microbicide vaginal rings [54–56].

2.4.2. Vaginal rings for HIV prevention under development

Numerous vaginal rings are currently under development for preventing the sexual transmission of HIV. Some examples are described below.

Malcolm *et al.* developed matrix silicone elastomer vaginal rings containing Maraviroc and CMPD167. *In vitro* release tests showed a controlled release of both drugs over 28 days. Their concentrations in vaginal fluid in a 28-day study in rhesus macaques were around 10^6-fold higher than the 50% inhibitory concentrations for inhibiting a simian/human immunodeficiency virus in macaque lymphocytes *in vitro* [58].

A core-matrix vaginal ring for preventing HIV-1, HSV-2 (herpes simplex virus-2), HPV (human papilloma virus) and unwanted pregnancy was proposed by Ugaonkar *et al*. MIV-150 and zinc acetate (anti-HIV drugs), carrageenan (also for HPV) and Levonorgestrel (contraceptive) were included in this multipurpose dosage form. MIV-150 and Levonorgestrel diffused from the EVA hydrophobic matrix of the ring while zinc acetate and carrageenan diffused through a pore from the hydrophilic core. The drugs were released *in vitro* over 94 days, and up to 28 days of the study period in rhesus macaques, showing effectiveness for virus protection and contraception [59].

A segmented dual-reservoir PU vaginal ring containing Tenofovir and Levonorgestrel was formulated by Clark *et al.* to protect women against HIV and unwanted pregnancy. The rings comprised a 10- or 20 mm-long polyether urethane-based reservoir segment containing 1.3 wt% Levonorgestrel surrounded by a 100 μm thick rate-controlling membrane also of polyether urethane. Tenofovir was loaded as glycerol paste in a hydrophilic PU reservoir segment separated from the Levonorgestrel segment by PU caps to prevent it from diffusing into the Tenofovir reservoir. The rings formed by the 20 mm-long segment of Levonorgestrel released both drugs in vitro over 90 days [60].

Study	Phase	Formulation	Reference
MTN-013/IPM 026	I	Silicone elastomer matrix-type vaginal ring loaded with 25 mg Dapivirine and 100 mg Maraviroc	[65]
2013-329	I	Polyurethane Tenofovir Disoproxil Fumarate vaginal ring	[66]
The Ring Study/IPM 027	III	Silicone elastomer matrix-type vaginal ring containing 25 mg Dapivirine	[67]
ASPIRE/MTN-020	III	Silicone elastomer matrix-type vaginal ring containing 25 mg Dapivirine	[68]

Table 4. Vaginal rings for HIV prevention under clinical investigation.

Srinivasan *et al.* described a pod-vaginal ring containing 65 mg of Tenofovir Disoproxil Fumarate and 68 mg of Emtricitabine and tested it in female pigtailed macaques. The ring was made of silicon elastomer and included three poly(vinyl alcohol) coated-pods of each drug and one delivery channel per pod. All the animals treated with the ring were protected against the infection compared to the control group, despite the exposure to simian/human immunodeficiency virus. The drugs were also released in a sustained manner and maintained effective levels over 4 months [61, 62].

Murphy *et al.* formulated rod insert silicone elastomer vaginal rings to release the candidate antiretroviral peptides T-1249 and JNJ54310516-AFP (JNJ peptide). The drugs were contained in a hydrophilic excipient—sodium chloride, sodium glutamate, lactose or zinc acetate at different concentrations—to form the peptide-loaded rod inserts. *In vitro* release tests showed the sustained release profiles that could be achieved with these rings and which may be related to the type of hydrophilic excipient and its swelling properties [63].

Vaginal rings based on biosoluble acacia gum and a non-biodegradable hydrogel of 2-hydroxyethyl methacrylate (HEMA) to release one or two anti-HIV drugs were studied by Saxena *et al*. Five types of rings were formed, namely acacia gum rings with Dapivirine, Boc-lysinated-betulonic acid (Boc-LBA) or a combination of TMC120 (Dapivirine) and PMPA (Tenofovir), and HEMA rings with Zidovudine or Dapivirine and Tenofovir. The results of the drug release assays showed drug levels greater than the minimum dose required to inhibit the virus, which were sustained for at least 15 days for acacia gum and 28 days for HEMA rings [64].

Table 4 shows some of the clinical trials on vaginal rings for HIV prevention.

3. Authors' conclusions and future perspectives

HIV and AIDS are huge health problems today, and especially in women in developing countries. Current systems for preventing transmission are generally beyond their reach, so it is essential to develop new strategies, among which an interesting option is the use of topical vaginal formulations as prophylaxis .

The vaginal route of administration has various complexities, and intense research is underway around the world to find the formulation that best meet the requirements. Vaginal gels and tablets are easy to manufacture and administer, but patients report comfort issues such as substantial leakage after administration. Vaginal rings overcome this problem, but are more expensive and difficult to manufacture. Vaginal leakage is also reduced by vaginal films, which are cheap and easy to manufacture, but these tools are still fairly novel and require more knowledge and technical improvements in the production process.

We therefore consider that sustained-release formulations may represent the most attractive option, since greater compliance is crucial to ensure the effectiveness of the microbicide. Although vaginal rings are the most suitable formulation for sustained release, vaginal films and tablets are also being investigated to obtain longer protection times, and are seen as promising platforms for the inclusion of microbicides to prevent the sexual transmission of HIV.

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